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Docosahexaenoic Acid (DHA) Supplementation of Orange Juice Increases Plasma Phospholipid DHA Content of Children

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ABSTRACT

The major dietary source of docosahexaenoic acid (DHA) is fish, which is not widely consumed by children. There is concern, therefore, that children may not receive adequate DHA and so might benefit from dietary supplementation. The aim of the present study was to evaluate the effects of providing a supplement of microencapsulated algal DHA in juice. We assessed the effects of two levels of DHA supplementation on the plasma phospholipid DHA content of healthy 4- to 6-year-old and 7- to 12-year-old children who were randomly assigned to consume 180 mL juice containing either 50 mg (lower dose) or 100 mg (higher dose) DHA daily for 6 weeks. Plasma phospholipid DHA content (mole % of total fatty acids) was measured before and after 6 weeks of daily juice consumption. Because there are no data for plasma phospholipid DHA content in healthy children, data were compared to that of breastfed infants. At baseline, plasma phospholipid DHA content was lower in both age groups and dose groups than observed in breastfed infants. It increased significantly in both dose groups, but more so in the higher dose group of both age groups ($P<0.05$, overall mean±standard deviation: 3.72±0.66 vs 4.64±0.77); reaching levels similar to or greater than content of breastfed infants. Thus, DHA supplementation of juice at either 50 mg/day or 100 mg/day for 6 weeks was effective in increasing plasma phospholipid DHA contents of children. *J Am Diet Assoc. 2009;109:708-712.*

The 18-carbon fatty acids, linoleic acid (LA, 18:2n-6) and α-linolenic acid (ALA, 18:3n-3), cannot be synthesized by mammals. Hence, these fatty acids as well as some of their metabolites, are considered essential fatty acids. Chief among the metabolites of these fatty acids are the n-6 fatty acids, arachidonic acid (ARA, 20:4n-6) and docosapentaenoic acid (22:5n-6), which are synthesized from LA; and the n-3 fatty acids, eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), which are synthesized from ALA.

In theory, because infants can convert LA and ALA to the longer chain, more unsaturated fatty acids (1), a balanced intake of LA and ALA should result in adequate amounts of all n-6 and n-3 polyunsaturated fatty acids (PUFA). However, while plasma and tissue levels of n-6 PUFA (ARA and docosapentaenoic acid) are usually adequate, plasma and tissue levels of n-3 PUFA, particularly DHA, are often low. This probably reflects modern diets, which are high in LA but contain considerably less ALA. Consequences of this imbalance are not known with certainty, but cognitive and visual function deficits of infants who receive a dietary source of ALA, but not DHA are well-documented (2). Potential consequences of low plasma n-3 PUFA content in children are not well-known; however, data suggest that n-3 PUFA may play a role in cognitive function and attention in children and psychiatric disorders in adults (3-5).

Fatty fish are among the richest dietary sources of EPA and DHA. However, fish intake by children under 14 years of age is only about half a serving per week (6). Further, while more than one-third of US children take multivitamin supplements regularly, few take DHA supplements (7).

Multiple organizations have established recommended levels for n-3 or fatty fish consumption to meet essential fatty acid needs. The dietary reference intake of n-3 PUFA is 0.6% to 1.2% of total calories; that for n-6 PUFA is 5% to 10% of total calories for adults and children over age 4 years of age (8). The 2005 Dietary Guidelines for Americans recommend that individuals, "Keep total fat intake between 20 and 35 percent of calories, with most fats coming from sources of polyunsaturated and monounsaturated fatty acids, such as fish, nuts, and vegetable oils" (9). The US Dietary Guidelines Advisory Committee recommends eating fatty fish twice weekly, aiming for approximately 8 oz per week (10). The American Heart Association and the National Academy of Sciences also promote consumption of fatty fish at least twice weekly (11,12). Finally, a recent position paper by the American Dietetic Association (13) recommends including two servings (4 oz per serving) per week of fatty fish high in n-3 fatty acids.

However, fish consumption is not without risks. The Environmental Protection Agency and the US Food and Drug Administration recommend that children as well as

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pregnant and lactating women avoid large predatory fish, which may contain high levels of methylmercury (12).

Because most children do not readily eat fish, an alternative source of DHA may be desirable. One possibility is to incorporate a microencapsulated algal oil high in DHA into a healthful food or beverage that is widely consumed (eg, orange juice). However, the amount of DHA needed, if any, is not clear. To further complicate the issue, there is no consensus concerning the desirable plasma phospholipid DHA content of children beyond infancy.

There are no long-term data in healthy children regarding the intake of foods or beverages supplemented with DHA. However, Birch and colleagues (14) determined that supplementation of term infant formula with DHA and ARA continued to support visual acuity and intelligence maturation similar to breastfed infants at 4 years of age. Data regarding cognitive function outcomes with DHA-supplemented foods beyond this age for healthy children do not exist. Thus, it is necessary to directly determine the effects of different intakes of DHA on plasma fatty acid patterns of children consuming the product containing DHA.

The primary objective of the study reported here was to determine plasma phospholipid DHA contents of 4- to 12-year-old children before and after a 6-week course of either 50 mg/day or 100 mg/day algal-based DHA in orange juice. Because there are no data for plasma phospholipid DHA content in healthy children, these values were compared with previously reported plasma phospholipid DHA contents of breastfed infants (15) and breastfeeding women supplemented for 4 months with 200 mg/day DHA (16).

METHODS

Healthy 4- to 12-year-old children were recruited by public advertising and word of mouth. Subjects were eligible for enrollment if they had no underlying health conditions that affected fat absorption, had a body mass index (calculated as kg/m^2) between the 5th and 95th percentile for age and sex, were not allergic to or intolerant of orange juice, and were not taking n-3 fatty acid supplements.

Children who qualified were assigned randomly by age (4 to 6 years and 7 to 12 years) and sex to receive 180 mL orange juice daily for 6 weeks supplemented with either 50 mg (lower dose) or 100 mg (higher dose) DHA. By 6 weeks of supplementation, subjects would be expected to have achieved a steady-state level of plasma phospholipid DHA content (17).

The DHA source was a single-cell oil derived from the algae, *Schizochytrium* sp. It was added to the orange juice by the manufacturer (Coca-Cola North America, Apopka, FL). The 50-mg and 100-mg DHA dose levels were chosen to reflect current levels of other foods and beverages supplemented with n-3 fatty acids, which typically range from 20 to 400 mg per serving.

Fasting plasma phospholipid fatty acid contents were determined at baseline and after 6 weeks. Blood was obtained in ethylene diamine tetraacetic acid–containing tubes and plasma was separated by centrifugation and stored at −70°C until analysis. Plasma lipids were extracted as described previously (18) and the phospholipid fraction was separated by one-dimensional thin-layer chromatography (17). Methyl esters of the component fatty acids of the phospholipid fraction, prepared with boron trifluoride-methanol (19), were quantified by gas-liquid chromatography (Varian 3500, Varian Inc, Walnut Creek, CA) in the presence of 17:0 and 21:0 internal standards. The amount of each fatty acid in the plasma phospholipid fraction is expressed as the mole percentage of the total fatty acids.

An essential fatty acid–specific food frequency questionnaire (FFQ) was administered to parents to assess typical DHA and EPA intakes of their children during the past month (20). Study calendars were provided to evaluate compliance by having subjects write a checkmark for each day they consumed the product and return them at the end of the study along with any unopened bottles of juice. Acceptability of the juice was assessed at the final visit by a taste questionnaire.

Baseline and 6-week fatty acid data of the two age groups were analyzed separately by *t* tests and, if no difference between groups was found, data were combined to simplify the interpretation of results. Differences between baseline and 6-week data were determined by paired *t* tests (SPSS, version 15, 2006, SPSS Inc, Chicago, IL). The Institutional Review Board of Baylor College of Medicine and Affiliated Hospitals approved the protocol and written informed consent was obtained from parents. Written assent was obtained from children who were aged 7 years or older.

Table 1. Characteristics of 4- to 12-year-old children receiving juice supplemented with docosahexaenoic acid

Characteristic	Lower dose (50 mg) (n=15)	Higher dose (100 mg) (n=16)
Sex	7 male; 8 female	8 male; 8 female
	← *mean*± *standard deviation* →	
Age (y)	7.1±2.2	7.5±2.8
Weight (kg)	26.0±10.5	26.2±10.1
Height (cm)	122.6±16.1	125.6±17.2
BMI[a]	16.6±2.3	15.9±2.3
Fish intake (servings/wk)	1.1±1.0	0.8±0.7
DHA[b] intake (mg/day)	56.2±30.6	55.3±33.3
EPA[c] intake (mg/day)	33.8±21.9	33.4±21.4
	← *n* →	
Ethnicity		
White	4	5
Hispanic	3	2
African-American	6	9
Asian	2	0

[a]BMI=body mass index (calculated as kg/m^2).
[b]DHA=docosahexaenoic acid.
[c]EPA=eicosapentaenoic acid.

RESULTS

Selected characteristics of the two groups are summarized in Table 1. By design, half of the subjects in each age group (four males and four females) received 50 mg/day DHA and the other half received 100 mg/day. Of the 32

Table 2. Fatty acid pattern of plasma phospholipid fraction (mole % of total fatty acids) of 4- to 6- and 7- to 12-year-old children at baseline and after 6 weeks of daily docosahexaenoic acid supplementation of 50 mg (lower dose) or 100 mg (higher dose)

	Lower Dose		Higher Dose	
Fatty acid	**Baseline**	**6 Weeks**	**Baseline**	**6 Weeks**
	← *mean± standard deviation* →			
Total saturated	43.13±1.79	42.91±2.16	43.36±1.70	42.60±1.04
4- to 6-year-olds	42.20±1.07	43.41±2.75	43.77±2.27	42.67±1.13
7- to 12-year-olds	44.07±1.93	42.33±1.15	42.96±0.79	42.53±1.02
Total monounsaturated	13.67±1.70	13.21±1.07	13.2±1.33	12.70±1.53
4- to 6-year-olds	13.85±1.75	13.50±0.88	13.55±1.26	12.36±1.89
7- to 12-year-olds	13.48±1.74	12.88±1.23	12.93±1.40	13.04±1.08
Total n-6	39.30±2.74	39.02±2.81	38.85±1.88	38.88±1.43
4- to 6-year-olds	39.95±2.27	38.24±3.21	38.32±1.91	39.02±1.89
7- to 12-year-olds	38.64±3.16	39.92±2.15	39.38±1.82	38.73±0.87
18:2n-6, linoleic acid	23.46±2.55	23.28±2.49	23.29±2.60	23.59±1.70
4- to 6-year-olds	23.44±2.29	22.54±2.85	22.49±2.70	23.43±1.87
7- to 12-year-olds	23.47±2.95	24.12±1.83	24.08±2.39	23.75±1.64
20:4n-6, arachidonic acid	11.42±1.87	11.55±2.34	11.44±1.93	11.03±1.46
4- to 6-year-olds	11.94±2.19	11.45±2.36	11.71±1.68	11.42±1.59
7- to 12-year-olds	10.90±1.45	11.66±2.49	11.17±2.23	10.65±1.31
22:5n-6, docosapentaenoic acid	0.61±0.18	0.74±0.16	0.58±0.12	0.72±0.13
4- to 6-year-olds	0.65±0.22	0.81±0.16	0.66±0.11	0.82±0.10
7- to 12-year-olds	0.57±0.12	0.66±0.12	0.50±0.05	0.63±0.09
Total n-3	3.89±0.85	4.84±0.70	4.30±0.74	5.83±0.85
4- to 6-year-olds	3.99±0.74	4.85±0.57	4.36±0.57	5.95±0.86
7- to 12-year-olds	3.78±0.99	4.87±0.95	4.23±0.91	5.70±0.87
18:3n-3, α-linolenic acid	0.20±0.06	0.20±0.09	0.18±0.10	0.14±0.06
4- to 6-year-olds	0.19±0.05	0.21±0.12	0.17±0.09	0.13±0.06
7- to 12-year-olds	0.21±0.06	0.18±0.03	0.20±0.11	0.15±0.06
20:5n-3, eicosapentaenoic acid	0.23±0.13	0.17±0.20	0.38±0.28	0.30±0.22
4- to 6-year-olds	0.27±0.09	0.25±0.26	0.51±0.33	0.41±0.21
7- to 12-year-olds	0.20±0.15	0.09±0.02	0.26±0.16	0.18±0.17
22:6n-3, docosahexaenoic acid	2.55±0.67	3.72±0.66*	2.77±0.78	4.64±0.77*†
4- to 6-year-olds	2.59±0.54	3.69±0.57*	2.74±0.65	4.67±0.75*†
7- to 12-year-olds	2.50±0.81	3.80±0.86*	2.80±0.93	4.61±0.84*†

*$P<0.05$, 6 weeks vs baseline.
†$P<0.05$, higher dose vs lower dose.

enrolled subjects, 1 of the 7- to 12-year-old males who received the 50 mg/day supplement refused to allow a poststudy venipuncture and no data from this subject are included. Weight, height, and body mass index differed between the two age groups as expected. There were no significant differences in any of the subject characteristics between the two dose groups.

The fatty acid pattern of the plasma phospholipid fraction is summarized in Table 2. There was no difference in the content of any fatty acid between the two age groups; thus, data are presented as the overall mean. There was no difference in plasma phospholipid content of any fatty acid between the two dose groups at baseline; but after 6 weeks of supplementation, plasma phospholipid DHA content of both groups was higher (lower dose: 2.55±0.67 vs 3.72±0.66; $P<0.05$ [mean for both age groups]; higher dose: 2.77±0.78 vs 4.64±0.77; $P<0.05$ [mean for both age groups]) with the higher dose group increasing more than the lower dose group ($P<0.05$). The plasma phospholipid content of no other fatty acid differed between dose or age groups, including ARA, which in some studies is decreased by DHA (21,22).

Based on the FFQ, the children in both age groups habitually consumed about one serving of fish per week (1.0±0.95 for 4- to 6-year-olds and 0.8±0.7 for 7- to 12-year-olds). This equates to 58±36 and 53±27 mg DHA per day and 36±25 and 31±18 mg EPA per day for the 4- to 6-year-olds and 7- to 12-year-olds, respectively.

The completed study calendars indicated that compliance with juice consumption was excellent. For all subjects, juice was consumed for 96%±6% of the study days. The relatively short duration of the study, the ease of single-serving containers, and the acceptance of taste (described as either "good" or "very good" [4 or 5 on a 1 to 5 scale] by 93% of the subjects) undoubtedly contributed to the excellent compliance. The stability of DHA-fortified juices was evaluated by the juice manufacturer. DHA concentration was determined by gas chromatography flame ionization detector following acid catalyzed transesterification to the methyl ester and sensory tests were

performed using standardized questionnaires. DHA concentration and sensory profiles of the DHA-supplemented juice were determined to be stable during the 9-week shelf life.

DISCUSSION

In both 4- to 6-year-olds and 7- to 12-year-old children, a daily 180 mL serving of orange juice supplemented with 50 or 100 mg DHA resulted, after 6 weeks, in a higher DHA content of the plasma phospholipid fraction. Supplementation with 50 mg/day led to a 40% to 50% higher phospholipid DHA content compared to baseline, and supplementation with 100 mg/day led to a 65% to 70% higher plasma phospholipid DHA content compared to baseline. Neither dose affected plasma phospholipid ARA content or content of any other fatty acid. What should be considered desirable in children of this age group is not clear.

Based on the FFQ, our subjects consumed approximately 18% of the amounts of DHA and EPA recommended by the US Dietary Guidelines Advisory Committee (10). This is not solely a result of low overall fish intake; these children's major source of dietary fish was fish sticks, which are often made from fish with low n-3 fatty acid content. Although to date the FFQ has only been validated in adults, the findings are consistent for children in the United States (6). Furthermore, to our knowledge, it is the only FFQ specific to n-3 PUFA available at this time.

The National Academy of Sciences has recommended more studies of EPA and DHA supplementation of children, particularly those with behavioral disorders (12). However, in order to interpret data from children with developmental disorders, it is necessary to know if differences exist between healthy children and those with such disorders. Currently, the only plasma phospholipid DHA content data for 6- to 12-year-old children are from children with attention-deficit/hyperactivity disorder, a proposed potential consequence of low plasma phospholipid DHA content. Baseline plasma phospholipid DHA content of these children was ~30% lower (~1.9 mol %) (3) than is reported here and may not represent optimal levels for healthy children without attention-deficit/hyperactivity disorder.

Few data regarding DHA supplementation and plasma phospholipid DHA content exist for healthy children over ~18 months of age. Therefore, we compared contents observed in healthy 4- to 12-year-old children with the phospholipid DHA contents observed in 4-month-old exclusively breastfed infants (3.6% of total fatty acids) (15) and DHA-supplemented breastfeeding women (3.4% of total fatty acids) (16). Plasma phospholipid DHA content of the 4- to 12-year-old children reported here were lower than that of term infants and breastfeeding women at baseline but, after 6 weeks of supplementation, differed only minimally. In addition, plasma phospholipid ARA content did not differ significantly from that of breastfed infants (13.8% of total fatty acids) (15).

Other DHA supplementation studies in children include a recent study evaluating fish oil supplementation at 15 mg/kg body weight in children with phenylketonuria (23). These patients had relatively low plasma phospholipid DHA content at baseline (2.37%±0.1%), secondary presumably to the low n-3 fatty acid content or their habitual diet but, after 3 months of supplementation, plasma phospholipid DHA content was considerably higher (7.05%±0.24%). The authors noted that the large increase in plasma phospholipid DHA content after supplementation might indicate that the dose of fish oil, three to five times the amount that was evaluated by us, was excessive. However, optimal contents for children with or without phenylketonuria are not established.

Studies in adults also have shown increases in plasma phospholipid DHA content after DHA supplementation with either capsules or fortified foods (24,25). With respect to the latter, n-3–fortified eggs have been available for some time and the total number of n-3–fortified products available is increasing; currently, n-3–fortified bread, cereal, pasta, yogurt, and even chocolate, are available.

Because brain turnover continues throughout life, much attention has focused on the role of n-3 PUFA in dementia and Alzheimer's disease. Findings from the Framingham Heart study showed a 50% lower risk of developing Alzheimer's disease with consumption of more than two servings of fish per week (26). DHA supplementation also has been shown to improve cognitive dysfunction associated with mild Alzheimer's disease (27,28). Based on the available data from infants and adults, there may be benefits of n-3 PUFA supplementation throughout the entire life cycle, including childhood and adolescence. While improved visual acuity, brain development, and verbal intelligence quotient have been reported in infants consuming infant formula supplemented with DHA and ARA, it is unclear what benefits would be manifested in older children.

CONCLUSIONS

The DHA-supplemented juice in this study was well-accepted and proved to be an effective means of enhancing DHA intake of children and achieving a higher plasma phospholipid DHA content. However, further studies are needed to determine if these changes are accompanied by functional benefits in healthy children. Substantial further research is necessary to evaluate potential benefits, particularly in the area of improved attention. Data regarding the effect of DHA-supplemented juice on pediatric plasma phospholipid DHA contents would be helpful to registered dietitians and other food and nutrition professionals, especially in light of the low fish intake among children.

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